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Patents ADP number *(if you know it)*: 7738530002

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4. Title of the invention: Tissue Rejection

5. Name of your agent *(if you have one)*:
CRUIKSHANK & FAIRWEATHER
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TISSUE REJECTION

The present invention relates to a preparation for use in the evaluation of the risk of tissue rejection of donor tissues. The invention further relates to preparations for use in suppressing tissue rejection, and still further to methods of assessing and suppressing tissue rejection of donor tissues.

Transplantation is now the preferred treatment for cases of chronic renal failure. There is however a large disparity between the number of potential transplant recipients and the number of available donor kidneys. This disparity is also evident in all other transplant cases. Failure of transplanted or grafted tissues (the term 'tissues' as used herein will be understood to refer equally to cells and organs) is therefore of extreme clinical, economic, and emotional significance on recipients of donor tissue. In terms of kidney transplants, chronic allograft nephropathy (CAN) represents the most common cause of graft loss, once patient death has been accounted for. Unfortunately, the pathogenesis of CAN, at present, remains poorly understood and little progress has been made in reducing its occurrence. A better understanding of the pathogenesis of CAN may be expected to

yield new light on how to reduce the occurrence of CAN (Halloran et al, 1999).

Previous hypotheses to explain the pathogenesis of CAN have concentrated on possible immune-based explanations. However, these now appear unlikely, since the majority of risk factors for CAN are non-immune, and the widespread introduction of new immunosuppressants in the 1980s did not lead to a significant drop in the occurrence of CAN (Paul, 1999).

It is among the objects of embodiments of the present invention to provide preparations for reducing the occurrence of CAN in donor tissues. This is achieved, in part, by the surprising findings of the present inventors that a number of senescence-associated genes (SAGs) have been found to show altered expression in tissues susceptible to CAN.

It has been suggested that one possible cause of CAN may be accelerated ageing, where the accumulated burden of injury and age exhausts the ability of key cells to repair and remodel, and therefore to retain tissue integrity (Halloran et al, 1999). The present invention is based upon a model for CAN in which damage to the tissue leads to altered expression of genes involved in cellular

senescence, resulting in CAN. The particular genes which are implicated in cellular senescence are the telomere-binding proteins, as discussed below. It has been surprisingly found by the present inventors that specific telomere-binding proteins show increased expression in kidneys that have been subject to CAN when compared to normal tissues. Surprisingly also, the present inventors have also found decreased expression of a given SAG in tissues derived from a cadaveric donor, when compared to those derived from a living donor, or indeed those from living donors that have subsequently undergone CAN.

According to a first aspect of the present invention, there is provided a method of screening mammalian donor tissues for predisposition to rejection, the method comprising the steps of determining the level of expression of an endogenous telomere-binding protein in the donor tissue, and comparing the determined level to a reference level of expression, altered levels of expression in the donor tissue being indicative of a predisposition to rejection.

Of course, it will be appreciated that the present method alone does not enable a medical practitioner to definitively assess whether or not a given donor tissue is

suitable for transplant. Many other factors must be taken into consideration, for example, the age and general health of both the donor and the recipient, the urgency of the transplant, immunological factors, and so forth. The skilled person will be aware of the many relevant factors which will be considered. Nonetheless, the method of the present invention is intended to be a useful addition to the skilled person's other considerations and techniques.

Preferably the method is carried out on a donor tissue previously removed from a donor body. Alternatively this method may be carried out on a donor tissue post-transplant in order to provide data for the determination and suitability of therapeutic intervention or change of therapeutic regime.

The mammalian tissue may be human or animal tissue, such as porcine tissue.

In a particularly preferred embodiment the tissue being tested is renal tissue.

The method may comprise determining the expression level of one or more telomere binding proteins; preferably at least two, and more preferably at least three. This allows assessment to be made on the basis of a number of diagnostic proteins, to reduce the risk of false positives.

Preferably the telomere binding protein is selected from the group comprising G22P1, XRCC5, hPOT1, and SIRT2, and their homologues or analogues. These genes and their proteins are described and characterised in the publications listed in the OMIM database at:

G22P1 - http://www3.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?152690

XRCC5 - http://www3.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?194364

hPOT1 - http://www3.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?606478

SIRT2 - http://www3.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?604479

Where the telomere binding protein is one or more of G22P1, XRCC5, and hPOT1, and homologues thereof, an increase in expression of these proteins is taken as indicative of a predisposition to rejection. Where the telomere binding protein is SIRT2 or a homologue thereof, a decrease in expression of this protein is indicative of a predisposition to rejection in tissue taken from a cadaveric source.

Alternatively, another telomere binding protein or senescence associated gene may be selected; for example,

the protein or gene may be selected from the group comprising Rif1, Rif2, Rap1, SIRTs 1,3,4,5, Est1, Est2, TLG1, cdc13, A26, ATM, HDAC1, hSEP1, hTEP1, HuCds1, MYC, NEK2, p21, PIN2, TNKS, TERC, hTERT, TOP2A, TOP2B, TP53, TRF1, TRF2, WRN. Full details of such genes are given at the following location: http://www.genlink.wustl.edu/teldb/pteldb/pteldb3.html.

The step of determining the level of expression of the telomere binding protein may be conducted by means of detection of the protein with antibodies thereto; the antibodies may themselves be labelled in some way (for example, radiolabelling, fluorescent labelling, or the like), or the antibodies may be subsequently detected using additional reagents, for example in the form of an ELISA assay well known to those of skill in the art. Alternatively, the levels of expression of mRNA encoding the protein may be determined, using, for example, Northern blotting, polymerase chain reaction (PCR) detection, *in situ* hybridisation, microarray or macroarray technology, or the like.

The reference level of expression may be a predetermined reference level, or may be determined substantially at the same time as the donor tissue level of

expression; for example, from tissues of the recipient or of a control subject. Preferably the reference level is obtained from a healthy tissue sample.

According to a second aspect of the present invention, there is provided a kit for screening mammalian donor tissues for predisposition to rejection, the kit comprising reagents for determining the level of expression of an endogenous telomere binding protein in the donor tissue.

Preferably the kit comprises PCR primers for detection of mRNA encoding the telomere binding protein. Preferably the protein is selected from the group comprising G22P1, XRCC5, hPOT1, and SIRT2, or homologues and analogues thereof. Alternatively, another telomere binding protein or senescence associated gene may be selected; for example, the protein or gene may be selected from the group comprising Rif1, Rif2, Rap1, SIRTs 1,3,4,5, Est1, Est2, TLG1, cdc13, A26, ATM, HDAC1, hSEP1, hTEP1, HuCds1, MYC, NEK2, p21, PIN2, TNKS, TERC, hTERT, TOP2A, TOP2B, TP53, TRF1, TRF2, WRN.

Alternatively, the kit may comprise antibodies to the telomere binding protein. The kit may further comprise detection reagents for detection of bound antibodies; alternatively or in addition the antibodies themselves may

be labelled to allow direct or indirect detection thereof. For example, radioactive or fluorescent labels may be used. A further alternative for the kit is that it may provide probes (such as DNA, RNA, or chimerical combinations of both or either) together with a further agent, for example a proteinaceous agent such as an antibody or other agent.

According to a further aspect of the present invention, there is provided a method of treatment of a mammalian donor tissue to reduce the risk of rejection, the method comprising the step of treating the tissue with an agent to enhance the activity, half-life or expression level of an endogenous telomere binding protein. Alternatively, or in addition, the tissue may be treated with an agent to enhance the effective functionality of the endogenous telomere binding protein.

It is believed that the increased expression levels of certain telomere binding proteins reflects increased levels of cell damage in rejection-susceptible tissues. Without wishing to be bound by theory, it is believed that as cellular DNA is damaged, existing telomere binding proteins are recruited away from the telomere to the site of the damage for repair purposes. Increased expression of these proteins results as the cell attempts to maintain

sufficient protein at the telomeres; this increased expression is detectable as discussed above. However, if insufficient protein is present, telomere damage and cell death or degradation may occur. Thus, further increasing the expression or otherwise assisting the functionality of these proteins may be expected to act to repair the damage and so reduce the risk of tissue rejection.

The donor tissue may be human or animal.

Conveniently the tissue is renal tissue.

Preferably the protein is selected from the group comprising G22P1, XRCC5, hPOT1, and SIRT2, and homologues or analogues thereof. The protein may instead comprise an active site homologous to that of G22P1, XRCC5, hPOT1, or SIRT2. Alternatively, another telomere binding protein or senescence associated gene may be selected; for example, the protein or gene may be selected from the group comprising Rif1, Rif2, Rap1, SIRTs 1,3,4,5, Est1, Est2, TLG1, cdc13, A26, ATM, HDAC1, hSEP1, hTEP1, HuCds1, MYC, NEK2, p21, PIN2, TNKS, TERC, hTERT, TOP2A, TOP2B, TP53, TRF1, TRF2, WRN.

One or more protein activity, expression levels or half-life may be enhanced; preferably two or more; more preferably three or more proteins are enhanced.

The enhancement of gene expression levels may be achieved via a number of strategies including : gene therapy; promoter / enhancer knock in; promoter / enhancer induction; gene replacement; and the like. These methods are not an exhaustive list of the full range of such techniques which typically are known to those of skill in the art.

According to a further aspect of the present invention, there is provided the use of a mammalian telomere binding protein in the preparation of a medicament for suppression of rejection of a donor mammalian tissue.

The tissue is preferably renal tissue.

The protein is preferably selected from the group comprising G22P1, XRCC5, hPOT1, and SIRT2, and homologues and analogues thereof. Alternatively, another telomere binding protein or senescence associated gene may be selected; for example, the protein or gene may be selected from the group comprising Rif1, Rif2, Rap1, SIRTs 1,3,4,5, Est1, Est2, TLG1, cdc13, A26, ATM, HDAC1, hSEP1, hTEP1, HuCds1, MYC, NEK2, p21, PIN2, TNKS, TERC, hTERT, TOP2A, TOP2B, TP53, TRF1, TRF2, WRN.

According to a still further aspect of the present invention, there is provided a non-human mammalian donor

tissue in which the expression of at least one endogenous telomere binding protein has been enhanced.

Such tissues may be useful in xenotransplantation, to lessen the risk of rejection of the donor tissue. Enhancement may be as a result of gene therapy, or transgenesis or molecular manipulation of cells subsequently used for nuclear transplantation. Promoter or enhancer knock in or induction element knock in are also applicable for these purposes.

These and other aspects of the present invention will now be described with reference to the accompanying drawings, in which:

Figure 1 shows the relative levels of expression of G22P1, XRCC5, hPOT1, and SIRT2 mRNA from different types of renal tissue; and

Figure 2 shows the results of a statistical analysis of the results of Figure 1.

Before discussing these Figures, for a better understanding of the invention, some further background detail will be given on the senescence of cells and the action of the relevant proteins.

Telomere instability

Telomeres are specialised structures found at the end

of eukaryotic chromosomes consisting of simple repetitive DNA, which in mammals comprises the sequence $(TTAGGG)_n$. Without specialised structures to cap telomeres and to replicate them during cell division, the telomeres gradually shorten over time. It is generally believed that shortening of telomere length through repeated cell divisions over time contributes to the ageing and senescence of somatic cells (reviewed in Shiels 1999).

The telomere capping function appears to be mediated by a combination of a unique tertiary structure and specific telomere binding proteins. Electron microscopy has shown that the mammalian telomere takes the form of a loop, termed the t-loop, created by the telomere DNA folding back on itself to form a lariat whose leading end is the telomeric 3' G strand overhang. This is envisaged as invading adjacent duplex telomeric repeats, thus creating a displacement loop (D-loop). Duplex DNA binding proteins are proposed to bind along the telomeric repeats of the t-loop, while a specialised DNA binding protein stabilises the D-loop lariat junction (Griffith et al., 1999)

Recently a protein capable of binding the single stranded G-rich extension found at the ends of all telomeres has been identified (Baumann and Cech, 2001).

This protein has been termed POT1 (Protection of Telomeres) and appears conserved in eukaryotes as diverse as yeast and humans. Deletion of the fission yeast pot1 gene produces immediate chromosome instability and rapid loss of telomeric DNA. The capping of telomeres thus appears to be crucial for avoiding chromosome instability and descent into cell death. In telomerase negative human somatic cells, telomeres may shorten with each cell division, finally reaching a critical level. However, cell death will not be triggered unless the telomere loses its capping proteins and becomes unstable. The state of the telomere (capped or uncapped) may also determine the degree of shortening which occurs with each cell division. Therefore telomere binding proteins may contribute directly to the internal mechanism which determines when the cell enters senescence.

Oxidative damage

Free radicals (ROS) are known to promote DNA damage and hence to contribute to the accumulation of damage which can promote cellular senescence. Oxidative metabolism is further known to promote production of ROS, and so promote senescence. Indeed, it has further been found that slowing metabolism of organisms, for example by restricting food

supply, prolongs life span to some extent. A number of observations provide an insight into how ROS may speed the entry of a cell into senescence.

Firstly, increased oxygen tension accelerates telomere erosion in replicating human fibroblasts *in vitro* (von Zglinicki and Schewe, 1995). Secondly, it has been observed that telomeres accumulated a significantly higher frequency of single-stranded overhangs, gaps and single-stranded breaks than the bulk of the genome when human fibroblasts were subjected to chronic oxidative stress (Peterson et al, 1998). Conversely, when human vascular endothelial cells were grown with an oxidation-resistant type of ascorbic acid, they showed a decrease in rate of telomere shortening by 52-62%, and an extension of cellular life span when compared to controls (Furumoto et al, 1998).

One possible explanation for this is that the G rich base repeat of telomeres is particularly susceptible to damage from ROS. This has been observed in lower eukaryotes where disruption of telomere structure occurs as a result of such damage. It is also possible that DNA damage to genomic DNA precipitated by ROS results in vital telomere binding proteins being recruited from the telomeres to the site of genomic DNA as part of the repair mechanism. This

would leave the telomeres exposed and uncapped, and thus more susceptible to instability.

Human *in vivo* evidence for an acceleration of telomere shortening in conditions of oxidative stress can be found be examination of telomeres of patients with respiratory chain disorders. These conditions are associated with an increased production of ROS due to faulty mitochondrial respiration, and telomeres in these patients were on average 1.5 kb shorter than those of controls (Oexle and Zwirner, 1997).

Molecular mechanisms underlying pathogenesis of CAN

The above observations are consistent with a hypothesis in which oxidative damage can lead to a senescent phenotype, indicating that such changes underlie the development of CAN. Such damage is particularly relevant to transplantation and grafting, where the donor tissue may have been subject to ischaemic and hypoxic stress, with consequent changes in metabolism and production of ROS. Further relevant observations include:

- The presence of senescent features such as telomere erosion in cloned sheep (Shiels et al. 1999a, b) despite the animal appearing physiologically normal is in keeping with a model of senescence based on

oxidative damage.

- Age related changes have been observed in mitochondrial DNA sequence and conformation (Kopsidas et al, 2000) along with an altered DNA end binding capacity, significantly, mediated through XRCC5 (Coffey and Campbell 1999).
- Mitochondrial xanthine oxidoreductase, a known cause of ischaemic reperfusion damage shows altered expression with age, resulting in elevated mitochondrial oxidative stress levels (Chung et al, 1999).
- *In vivo* murine gene expression analysis and *in vitro* models of interstitial fibrosis indicate specific perturbation of the genes for telomere binding proteins, anti oxidant enzymes, and ribosomal components, in common with senescing cells.
- Mutations altering the function of telomere binding proteins can result in progeria (Myung et al, 2000).
- Specific age related changes in the expression of genes involved in the formation of the cytoskeleton and the extracellular matrix are also observed in kidneys undergoing CAN (Linskens et al, 1995).

At a molecular level, much of the damage of a

prolonged cold ischaemic time will result directly from the ROS produced when hypoxic cells are reoxygenated. An increase in chronic rejection of transplants has been observed to correlate to prolonged cold ischaemic times.

ROS can accelerate cellular senescence directly by the mechanisms described above. Therefore the degree of cellular senescence already present within the donor tissue will become important, representing the degree of reserve that the cells have. Those cells that are nearer the end of their life span (for example, with shorter telomeres) will be more susceptible to damage from ROS and will enter senescence faster.

Without wishing to be bound by theory, the present inventors believe that oxidative stresses affecting transplant rejection result in changes in the production of ROS, and consequently the expression of telomere binding proteins. In particular, expression changes in G22P1, XRCC5, hPOT1, and SIRT2 have been investigated. These proteins are involved in the maintenance of telomere structure, DNA repair, and silencing of subtelomeric regions.

G22P1 and XRCC5 monomers form part of a complex that was originally identified as a major autoantigen in

patients with autoimmune diseases such as scleroderma. It is known to bind the ends of double-stranded DNA with high affinity and to be involved in repair of DNA by NHEJ. They are also known to be specifically co-located at the telomeres with SIRT2 and Rap1, and to play an important role in telomere maintenance in yeast. These products are known to interact with the Werner helicase protein, and are also responsible for the silencing of genes adjacent the telomere (Nugent et al 1998) and are found along with SIRT2 along this silenced region next to the telomere. In yeast, loss of the analogous heterodimer, Ku, from the telomere promotes loss of SIRT2, for example when Ku is recruited to repair a double stranded DNA break.

It is believed that the expression of telomere binding proteins is diagnostic for CAN and graft suitability. The present inventors suggest that DNA damage accrued pre-transplant results in substantial upregulation of G22P1 and XRCC5 expression, with concomitant telomere destabilisation as their respective gene products are recruited to DNA breaks elsewhere in the chromosome. Increase in hPOT1 expression occurs in an attempt to offset the destabilisation. Further, enhancement of expression of these proteins in the pre-transplant tissue would be

expected to reduce the risk of ROS damage to the cell, and hence lessen the risk of subsequent tissue rejection. Post-transplant intervention may also be possible to lessen the risk of subsequent damage and hence rejection.

Materials and Methods

RNA Isolation. Small frozen biopsy (10-50 mg) samples from human allografts were used to prepare RNA using Trizol (Sigma-Aldrich Co. Ltd, Poole, UK) following manufacturer's recommendations.

Samples were homogenised in Trizol using a PowerGen 125 Tissue Homogenizer (Fisher Scientific) using a test tube pestle. The homogenisation was performed until a homogeneous solution was obtained and very few visible tissue pieces were present. Samples were then incubated at room temperature for 5-10 minutes and the homogenate transferred to a centrifuge tube, and centrifuged at 12,000g (10,000 RPM on SS34) for 5-10 minutes at 4°C.

The upper layer was removed using a Pasteur pipette and 0.2 ml chloroform added per 1 ml Trizol reagent used initially. The tube was shaken vigorously for 15-30 seconds, then incubated at room temperature for 5 minutes. Samples were then spun at 12,000g for 15 minutes at 4°C. The upper aqueous phase, which contains the total RNA, was

removed and placed in a new centrifuge tube.

The RNA was precipitated by adding 0.5 ml isopropyl alcohol per 1 ml Trizol reagent used, and then incubated at room temperature for 10 minutes, followed by centrifugation at 12,000g for 10 minutes at 4°C. The supernatant was then decanted and the pellet washed once using 70% ethanol and spun at 7,500g (7,500 RPM in SS34) for 5 minutes at 4°C. The supernatant was again decanted and the pellet air dried briefly. The RNA pellet was then resuspended in 200 µl DEPC water, and quantified by spectrophotometry.

Slot blotting. The slot blot apparatus was assembled using Hybond N+ nylon membrane (Amersham, UK) as the solid support in accordance with the manufacturer's instructions, particular care being taken that no leakage occurred between wells before loading the RNA samples. This was tested using Orange G in sterile water as a marker dye. The wells were rinsed twice with 10 x SSC and fluid sucked through the manifold under vacuum. The vacuum was then turned off, and a further 50 ml of 10 x SSC added to each well. Two volumes of RNA loading buffer was added to each 5 µg sample of RNA, which were then denatured at 70°C for 10 minutes, and chilled on ice. RNA samples were then loaded into the wells already containing 10 x SSC and aspirated

through under vacuum. The wells were then rinsed twice with 200 µl of 10 x SSC and this drawn through under vacuum. The resulting slot blot was then rinsed in 2 x SSC and briefly air dried, then the RNA UV cross linked in place and stored at room temperature until required for hybridisation.

Hybridisation. This was performed at 65°C in 6 x SSC overnight with the respective probes labelled with α-^{32}P. Subsequent washing post-hybridisation was to a stringency of 0.2 x SSC at 65°C. The blot was then subjected to analysis using a Molecular Dynamics PhosphorImager. Statistical evaluation of signals was conducted using Microsoft Excel.

Generation of hybridisation probes. Probes were developed by RT-PCR of human mesangial cell RNA, using primers designed to amplify parts of the mRNA sequence of the gene of interest. Primers for hPOT1 were selected according to published data (Baumann and Cech, 2001). Primer sets for SIRT2, G22P1 and XRCC5 were developed from the respective GenBank sequence records using a specific algorithm provided by Vector NTI suite (Informax). Primer sequences are as follows:

1. XRCC5

Primer Sense: CTG AGG ACC GGC AAC ATG GT

Primer Antisense: CCA TCT TCC TTG CCA AGT GA

2. G22P1

Primer Sense: AAC ACG TCT CTT GCC AGG TC

Primer Antisense: CAC AAG TCT GGT GGT GGT GT

3. hPOT1

~~Primer Sense: GGA AGT GCA GCA GGG TTC AG~~

Primer Antisense: GGT TCT GCT TCC CTT TCA GT

4. SIRT2

Primer Sense: CCG CTA AGC TGG ATG AAA GA

Primer Antisense: GAC TGG GCA TCT ATG CTG GC

The level of gene expression was then measured by in-situ hybridisation with the appropriate amplicons.

Experiment 1

Consent was obtained to take a small biopsy (10-50 mg) of renal tissue from the allografts at transplantation or nephrectomy. RNA was extracted from this biopsy and 5 µg transferred by means of a slot blot onto Hybond N+ membrane. Probes were developed by RT-PCR of human mesangial cell RNA using primers as described above. The level of gene expression was then measured by *in situ* hybridisation with the appropriate α-^{32}P labelled probe. Phospho-images of the hybridised blots were then developed and analysed using ImageQuant technology.

A total of 4 samples from allografts with CAN, 4 from T_0 cadaveric donor biopsies, and 8 from T_0 living donor biopsies was analysed on multiple Northern slot blots. Comparative expression data from a representative blot is illustrated in Figure 1. This indicates that there was an increase in the expression of G22P1 and XRCC5 in the CAN samples when compared to T_0 living and cadaveric samples. Statistical analysis using a split-plot analysis of variance was undertaken to compare expression for each gene in a) T_0 living and T_0 cadaveric samples; and b) T_0 living and CAN samples, with comparison being made between the three biopsy classes for the four genes tested. The raw data from the analysis is shown in Figure 2.

Overall kidney type differences were assessed by comparison with the residual variation between samples and are significant at $p<0.01$. Gene differences were assessed by comparison with the residual variation within samples and are significant at $p<0.001$. Overall differences between kidney types with genes were assessed by comparison with a combination of between and within sample variation, and are significant at $p<0.05$. G22P1, XRCC5, and hPOT1 analysed within the respective groups showed significant differences in expression (at $p<0.05$). Surprisingly, there was also a

significant increase in expression of SIRT2 in T_0 living samples when compared to T_0 cadaveric, despite no difference being observed between T_0 living and CAN samples ($p<0.05$).

The increased expression of G22P1, XRCC5, and hPOT1 in CAN biopsies indicates that cellular senescence is a contributory factor in the pathogenesis of CAN. The reduction of SIRT2 expression in T_0 cadaveric samples when compared to T_0 living samples is interesting, and may reflect the mechanism by which oxidative damage promotes cellular senescence and therefore CAN, as this was not observed for the other three senescence associated genes. The observed differences in expression data do not appear to be correlated with the age of the donor organ, but rather are a specific reflection of senescence associated processes.

These results indicate that the G22P1, XRCC5, and hPOT1 genes are early markers for the development of CAN, and suggest a method for the screening of donor tissues prior to transplantation. For example, if features of cellular senescence, such as increased expression of G22P1 and XRCC5, are found in donor kidneys, it might be assumed that these grafts are more likely to develop CAN than those with low levels of expression of these genes. Routine

biopsies that indicated a sharp increase in the expression of G22P1 or XRCC5 and therefore the imminent development of CAN would contribute to the more precise management of the transplanted kidney, or to the suppression of the development of CAN. Further, the results of analysis of SIRT2 expression indicate that a decrease in SIRT2 expression in cadaveric samples may be prognostic for the future utility of cadaveric tissues. Enhancement of expression of all four genes may be expected to provide additional protein for cell and DNA maintenance and repair, and so prolong the effective lifespan of transplanted organs and reduce the risk of rejection.

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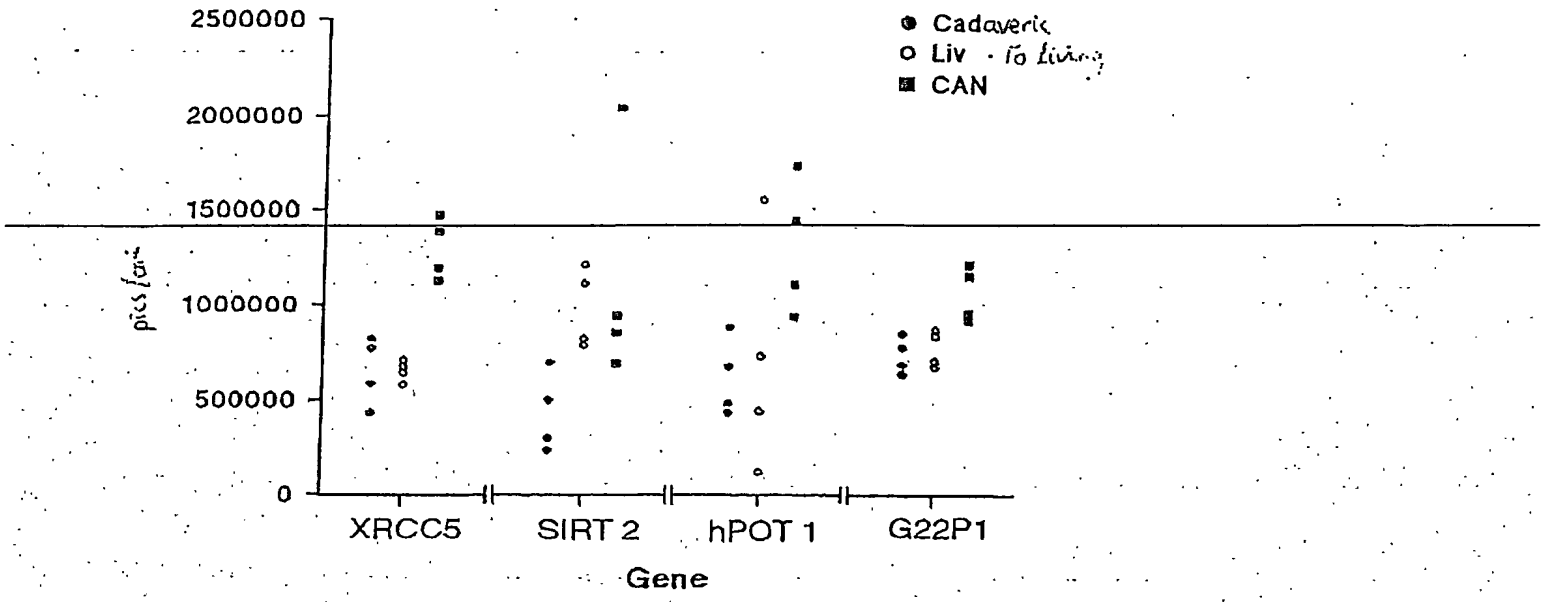
Figure 1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XRCC5
SIRT 2
hPOT 1
G22P1
Gene

Figure 2

***** Analysis of variance *****
Variate: SQRTsig

Source of variation	d.f	s.s	m.s	v.r	F pr.
Sample stratum					
Kidney type	2	191398	95699	11.92	0.003
Residual	9	72228	8025	2.09	

Samples.*Units* stratum

Genes	3	3240289	1080096	281.32	<.001
KidType.Genes	6	59590	9932	2.59	0.041
Residual	27	103664	3839		
Total	47	3667169.			

***** Tables of means *****
Variate: SQRTsig
Grand mean 505.3

Kidney type	Cadav	Live	Reject
	436.5	490.6	589.0

Genes	Gene1	Gene2	Gene3	Gene4
	924.3	350.1	506.9	240.1

KidType	Gene1	Gene2	Gene3	Gene4
Cadav	864.6	306.4	366.2	208.7
Live	882.5	308.0	563.6	208.2
Reject	026.0	435.8	590.8	303.5

*** Standard errors of differences of means ***

Table	KidType	Genes	KidType
	Genes		
rep.	16	12	4
s.e.d.	31.67	25.3	49.43
d.f.	9	27	31.64

The comparisons where there are significant differences (at $p<0.05$),
are reject > live & live = cadav for gene 1
reject > live & live = cadav for gene 2
reject = live & live > cadav for gene 3and reject = live & live = cadav for gene 4.

***** Tables of means *****

Variate: Signal

Grand mean 331772.

KidType	Cadav	Live	Reject
2	57252.	311656.	426409.

Genes	Gene1	Gene2	Gene3	Gene4
	861825.	126857.	275505.	62901.
KidType				
Cadav	749194.	95149.	140235.	44428
Live	780627.	94960.	320138.	50898.
Reject	1055656.	190461.	366141.	93378

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S. S.